PHYTOALEXIN INDUCTION IN RUBIACEAE

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(Received July 30, 1990; accepted February 4, 1991)

Abstract—Phytoalexin responses were measured by modified drop-diffusate and facilitated diffusion techniques after fungal inoculation of leaves of 32 Rubiaceae species from Brazilian forest and savanna. Such responses presented a trend similar to that previously observed for a broad sample of dicotyledonous plants and are more frequently positive for the more primitive (or slower growing) trees than for the advanced (or faster growing) herbs. Fifteen of these species analyzed during a one-year period showed that positive phytoalexin responses are stronger for the rainy (and hotter) than for the dry (and cooler) season. Species that contain relatively large quantities of phenolics gave invariably negative responses. Positive responses are not necessarily associated with the appearance of new substances within leaf tissue and are thus caused by inhibitins rather than by phytoalexins. These results are discussed recognizing that the tested plants are subject to the multifarious influences of their natural environment and of a possible conjugate-caused compartmentation of plant metabolites.

Key Words—Phytoalexins, fungitoxins, inhibitins, Rubiaceae, seasonal variations, leaf diffusates, leaf extractives, fungitoxin–phenol conjugation.

INTRODUCTION

Observation of phytoalexin induction is strongly influenced by the experimental techniques used. Application of the drop-diffusate (dd) procedure results in the

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1Taken in part from the MSc thesis presented by M.R.B. to Universidade de São Paulo (1988).
predominance of positive phytoalexin responses in cultivated (rather than in wild) species, while a modified facilitated diffusion (fd) procedure leads to comparable results for cultivated and wild species. Positive phytoalexin responses by both procedures are more common in summer, the rainy season, than in winter, the dry season. These correlations were encountered during a survey of phytoalexin formation involving 169 species belonging to 43 dicotyledonous families (Braga et al., 1986). Such broad sampling answered the main query of the investigation: the evaluation of the ubiquity of the phenomenon, but was of course not suited to reveal phylogenetic and environmental constraints.

It was the purpose of the present work to verify if morphologically closely related species could reveal clearly interpretable trends. To this end, sampling was reduced to 33 specimens of 32 species of the family Rubiaceae, substantially all of which had already been carefully marked and identified in a previous work (Braga et al., 1986). The number of species, even if minute in comparison with the ca. 7000 recognized species of the family, is nevertheless representative since it includes 44% and 48% of the species known to exist in the examined habitats, a forest (Jung-Mendagolli, 1984) and a savanna (Mantovani, 1983), respectively, of southeastern Brazil.

METHODS AND MATERIALS

Recently collected leaves from adult plants were used in all tests. The plants occur either in the Reserva Biológica, Instituto de Botânica, São Paulo, SP (forest) or in the Estação Ecológica e Experimental de Moji Guacu, Fazenda Campininha, SP (savanna) and are listed in Table 1. The data on climate were obtained from Instituto Astronômico e Geofísico, Universidade de São Paulo, SP (forest) or from Estação Ecológica e Experimental de Moji Guacu, SP (savanna).

All experimental details, including collection of leaves, nature and administration of Trichoderma pseudokoningii Rifai, the inducer fungus, and Cladosporium cladosporioides (Fresen) de Vries, the detector fungus by the modified drop-diffusate (dd) and facilitated diffusion (fd) techniques have been described in a previous paper (Braga et al., 1986). After the initial screening of 32 species for phytoalexin response, 15 individuals of 15 species (eight from forest and seven from savanna) were selected in order to follow up these responses during a one-year period. Phenolic content in leaf diffusates of 14 of these plants assayed in autumn was measured by the Folin-Denis procedure (Swain and Hillis, 1959), using phenol as the standard.

Fungitoxic substances were extracted from leaves of eight of these species by Keen's technique (Keen, 1978). Aliquots of extracts were submitted to thin-layer chromatography, using hexane–ethyl acetate–methanol (60:40:1) fol-